#### (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

# (19) World Intellectual Property Organization International Bureau



### 1 (2011) 1 (1)(1)(1) 1 (1)(1) 1 (1)(1) 1 (1)(1) 1 (1)(1) 1 (1)(1) 1 (1)(1) 1 (1)(1) 1 (1)(1) 1 (1)(1) 1 (1)(1)

# (43) International Publication Date 27 May 2004 (27.05.2004)

#### **PCT**

# (10) International Publication Number WO 2004/044246 A2

(51) International Patent Classification7:

C12Q 1/68

(21) International Application Number:

PCT/US2003/035919

(22) International Filing Date:

12 November 2003 (12.11.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: 60/425,620

12 November 2002 (12.11.2002) US

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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

[Continued on next page]

#### (54) Title: METHODS AND COMPOSITIONS FOR DETECTING TELOMERASE ACTIVITY

Providing a reaction tube that contains a first reaction mixture having a first primer and deoxynucleoside triphosphates in a top portion of the tube, a second reaction mixture having a second primer and a DNA polymerase in a lower portion of the tube, and a layer of wax separating the first reaction mix from the second reaction mixture 102 Adding a biological sample to the first reaction mixture and incubating the first reaction mixture under conditions suitable for a telomerase to produce an extension product from the first primer 104 Elongating the extension product 106 Admixing the extension product with the second reaction mixture by melting the wax layer 801 Amplifying the extension product by real-time PCR 110 Quantifying the amplified extension product using a control template 112

(57) Abstract: A method for determining telomerase activity using primer extension followed with real time PCR quantification is disclosed. The method of the present invention provides a rapid, sensitive and accurate measurement for telomerase activity in a biological sample. In one embodiment, the method includes the steps of: adding the biological sample to a reaction tube containing a first reaction mixture having a first primer and nucleoside triphosphates, a second reaction mixture having a second primer and a DNA polymerase, and a wax layer that separates the first reaction mixture from the second reaction mixture; incubating the biological sample with the first reaction mixture; admixing the extension product with the second reaction mixture; amplifying and quantifying the extension product using real-time PCR and a control template. In another embodiment, the detection method includes an in situ primer extension step that allows the production of the extension product within an intact cell. In this embodiment, the extension product can be preserved under appropriate conditions for an extended time before the completion of the quantification step.

#### WO 2004/044246 A2



(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

#### Published:

 without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



Rec'd PCT/PTO 16 MAY 2005
Inte mal Application No 16 MAY 2005
PCT/US 03/35919

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A. CLASSIFICATION OF SUBJECT MATTER 1PC 7 C12Q1/68 C12Q1/48							
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	European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Osborne, H					



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onal application No. PCT/US 03/35919

Box I	Observations where certain claims were found unsearchable (Continuation of item	1 of first sheet)			
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:					
1.	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:				
2.	Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed recan extent that no meaningful International Search can be carried out, specifically:	quirements to such			
з. [	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sent	ences of Rule 6.4(a).			
Box II	Observations where unity of invention is lacking (Continuation of item 2 of first she	et)			
This Inte	ernational Searching Authority found multiple inventions in this international application, as follows:				
	see additional sheet				
1.	As all required additional search fees were timely paid by the applicant, this International Search Reposearchable claims.	ort covers all			
2.	As all searchable claims could be searched without effort justifying an additional fee, this Authority did of any additional fee.	not invite payment			
з. 🗌	As only some of the required additional search fees were timely paid by the applicant, this International covers only those claims for which fees were paid, specifically claims Nos.:	al Search Report			
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4. X	No required additional search fees were timely paid by the applicant. Consequently, this International search to the invention first mentioned in the claims; it is covered by claims Nos.:	Search Report is			
	1-7, 15-18 and partially 20				
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Remark o	on Protest The additional search fees were accompanied by	the applicant's protest.			
	No protest accompanied the payment of additional	l search fees.			

### FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-7,15-18 and partially 20

A single-tube in vitro assay method for quantitative determination of telomerase activity in a cell sample using real-time PCR, and application of the method for monitoring the effectiveness of treatment of a patient.

2. claims: 8-14, 19 and partially 20

An in vivo assay method for quantitative determination of telomerase activity in a cell involving real-time PCR, and application of the method for monitoring the effectiveness of treatment of a patient.

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